

Laser Scanning Cytometry And Tissue Microarray Assessment of Osmoregulatory Capacity in Euryhaline Teleosts

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Abstract:

The size and number of mitochondria-rich chloride cells (CC) and the activity of Na^+/K^+ -ATPase are good indicators of osmoregulatory capacity in teleosts. In this study we have analyzed salinity-dependent changes in CC and Na^+/K^+ -ATPase using laser scanning cytometry (LSC) and tissue microarrays (TMAs) in tilapia (*Oreochromis mossambicus*) and killifish (*Fundulus heteroclitus*). Acute and gradual salinity acclimation regimens were designed and gill epithelium collected after 1, 2, and 5 weeks at fresh water, 1x seawater, or 2.4x seawater. Gill epithelial cells were dissociated and cell suspensions stained with DASPMI, followed by evaluation using a laser scanning cytometer. CC number and volume are proportional to external salinity, being lowest in FW and highest after 5 weeks in 2.4x SW. Tissue microarrays were constructed from fixed gill tissues and developed with antibody for Na^+/K^+ -ATPase to visualize CCs in tissue context and compare their characteristics with isolated CCs. Na^+/K^+ -ATPase per CC increases transiently after 1 week acute acclimation to 1x SW but returns to baseline within 5 weeks. Gradual acclimation to 2.4x SW also increases Na^+/K^+ -ATPase per CC but this increase is stable for at least 5 weeks. CC size in tissue-context did not correlate well with external salinity because of extensive basolateral membrane infoldings. Taken together, these data demonstrate the usefulness of laser scanning cytometry and tissue microarray analysis for assessing the osmoregulatory capacity of teleosts.